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ORIGINAL ARTICLE

Development and validation of a reversed-phase HPLC method for analysis of tetrahydrozoline hydrochloride in eye drop formulations

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Abstract A simple, precise, accurate, and stability-indicating method is developed and validated for analysis of tetrahydrozoline hydrochloride in eye drop formulations. Separation was achieved on a reversed-phase C_8 column (125 mm × 4.6 mm i.d., 5 μm) using a mobile phase consisting of acetonitrile/phosphate buffer of pH 3.0 (20:80, v/v) at a flow rate of 1.0 mL/min and UV detection at 240 nm. This method is validated according to United States Pharmacopeia requirements for new methods, which include accuracy, precision, selectivity, robustness, and linearity and range. This method shows enough selectivity, accuracy, precision, and linearity and range to satisfy Federal Drug Administration/International Conference on Harmonization regulatory requirements. The current method demonstrates good linearity over the range of 0.025–0.075 mg/mL of tetrahydrozoline with r^2 0.999. The average recovery of the method is 100.8% with a relative standard deviation of 0.47%. The degree of reproducibility of the results obtained as a result of small deliberate variations in the method parameters and by changing analytical operators has proven that the method is robust and rugged.

1. Introduction

Tetrahydrozoline is a derivative of imidazoline, it is an alpha agonist and its main mechanism of action is the constriction of conjunctival blood vessels. This serves to relieve the redness of the eye caused by minor ocular irritants. Tetrahydrozoline is used in eye and nasal drop formulations. Its structure is shown in Fig. 1.

The United States Pharmacopeia method for tetrahydrozoline analysis as raw material and in nasal and ophthalmic solutions is UV–vis spectrophotometry [1], which cannot detect impurities, degradation products, excipients, or preservatives present in the

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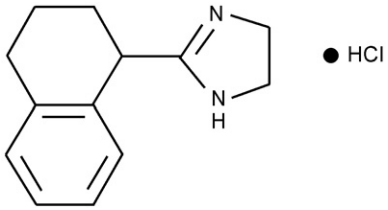

Figure 1 Structure of tetrahydrozoline hydrochloride.

pharmaceutical dosage forms. The British Pharmacopeia method for tetrahydrozoline raw material analysis is titration with perchloric acid [2], while pharmaceutical formulations of tetrahydrozoline are not included in the British Pharmacopeia. Formulations of tetrahydrozoline hydrochloride may contain preservatives e.g. benzalkonium hydrochloride that absorb in the same UV-region of tetrahydrozoline. Thus, UV–vis spectrophotometry cannot be used as selective and stability-indicating method for tetrahydrozoline analysis in these formulations. In this respect, a stability-indicating HPLC method is required for the analysis of tetrahydrozoline in pharmaceutical formulations (nasal and eye drops). Tetrahydrozoline hydrochloride was determined by HPLC in RP-mode [3] and in reversed-phase ion pair mode [4]. Tetrahydrozoline hydrochloride was also determined in combination with other ingredients: fluorometholone [5], ofloxacin and prednisolone acetate [6], antazoline [7], naphazoline [8], and in combination of its decomposition products [9]. The current work presents a reversed phase and stability-indicating method for analysis of tetrahydrozoline hydrochloride in eye drop formulations. The method is simple where reversed-phase-LC with isocratic elution and UV detection was used. Validation of the method was performed according to the requirements of United States Pharmacopeia for assay determination, which includes accuracy, precision (repeatability and intermediate precision (ruggedness)), selectivity, robustness, and linearity and range. Additionally, in order to meet the regulatory guidance of the Federal Drug Administration/International Conference on Harmonization (ICH) [10], tetrahydrozoline hydrochloride was degraded forcibly in acidic, basic, and strong oxidizing agent solutions.

2. Experimental

2.1. Chemicals

Acetonitrile HPLC grade was from J.T. Baker (NJ, USA). Potassium dihydrogen phosphate, triethylamine, phosphoric acid, hydrochloric acid, sodium hydroxide, and hydrogen peroxide were from Merck (Darmstadt, Germany). Tetrahydrozoline hydrochloride RS was from United States Pharmacopeia (Rockville, MD, USA).

2.2. Apparatus

HPLC system (Dionex system, USA) with a detector (PDA-3000, 08011275), equipped with a pump (LPG-3400A, 8007455), autosampler (WPS-300SL analytical 8008961), column compartment (TCC-3000, 8008864), and solvent rack (SR-3000) was employed during this study. The Chromeleon software was employed. The chromatographic analysis was performed on C_8 column (125 mm × 4.6 mm i.d., 5 μm) (Waters Corporation, Milford, Massachusetts, USA).

2.3. Standard solutions and HPLC conditions

Phosphate buffer was prepared by dissolving 1.0 g of potassium dihydrogen phosphate in 1000 mL of water, adding 3 mL of triethylamine, and adjusting the pH to 3.0 with dilute phosphoric acid solution. Diluent was prepared by adding 0.5 mL of phosphoric acid to 1000 mL of water. Filtered and degassed mixtures of acetonitrile and buffer (different volume fractions) were tested as mobile phases for tetrahydrozoline hydrochloride analysis. Different flow rates (1.0, 1.5, and 2.0 mL/min) were also tested. UV detection was performed at 240 nm and injection volume was 20 μL.

Stock standard solution of tetrahydrozoline was prepared by dissolving a quantity of tetrahydrozoline hydrochloride equivalent to 100.0 mg of tetrahydrozoline in 100.0 mL of diluent to obtain a solution having a known concentration of 1.0 mg/mL tetrahydrozoline.

Nominal (working) standard solution was prepared by diluting 5 mL of stock standard solution to 100 mL diluent to obtain a solution having a known concentration of 0.05 mg/mL tetrahydrozoline.

Nominal solutions of the formulated tetrahydrozoline eye drops were prepared by mixing a volume of the eye drops (5.0 mL) equivalent to 2.5 mg of tetrahydrozoline in 50 mL of diluent.

3. Results and discussion

3.1. Method development

Preliminary studies involved trying C_8 and C_{18} reversed-phase columns and testing several mobile phase compositions were conducted for the separation of tetrahydrozoline hydrochloride with good chromatographic parameters (e.g. minimized peak tailing and good symmetry). A C_8 column (5 μm, 125 mm × 4.6 mm i.d.) as a stationary phase with a mobile phase of acetonitrile/phosphate buffer pH 3.0 (20:80, v/v) at a flow rate of 1.0 mL/min and a detection wavelength of 240 nm afforded the best separation of tetrahydrozoline.

3.2. Method validation

After method development, validation of the current test method for tetrahydrozoline hydrochloride was performed in accordance with United States Pharmacopeia requirements for assay determination (category-I: analytical methods for quantitation of active ingredients in finished pharmaceutical products) which include accuracy, precision, selectivity, robustness, and linearity and range [10–14].

3.2.1. Linearity and range

To evaluate linearity of the method, five calibration standards of tetrahydrozoline containing 0.025, 0.0375, 0.05, 0.0625, and 0.075 mg/mL were analyzed. A plot of peak areas versus tetrahydrozoline concentration was linear in the range from 0.025 to 0.075 mg/mL of tetrahydrozoline with a correlation coefficient of 0.999. This result demonstrates linearity of this method over the specified range.

3.2.2. Accuracy and percentage recovery

Accuracy of the method was studied by preparing the placebo of the drug formulation according to the formulation procedure. To the required quantity of placebo, a known quantity of tetrahydrozoline with the same proportion as in the drug formulation was added to get three concentrations (0.025, 0.050, and 0.075 mg/mL of tetrahydrozoline). Results have shown that the recovery of tetrahydrozoline is within 99.8.0–101.3%, and the RSD is lower than 1.0% (Table 1).

3.2.3. Precision

3.2.3.1. Repeatability. Repeatability of the method was evaluated by calculating the RSD of the peak areas of six replicate injections for the standard concentration (100%) of tetrahydrazoline, which was found to be 0.27%. Furthermore, the RSD of the peak areas of the recovery data analyzed in accuracy study (see Section 3.2.2) for each level was calculated, and it was found to be less than 1.0% for each level (0.35%, 0.26%, and 0.62% for 50%, 100%, and 150%, respectively), as shown in Table 1. These results show that the current method for tetrahydrozoline analysis is repeatable.

3.2.3.2. Intermediate precision (ruggedness). Intermediate precision (also called ruggedness) of the method was also evaluated by analyzing six samples of tetrahydrozoline by two analysts in the same laboratory using different HPLC systems. Results of this study showed that the RSD of the percentage of tetrahydrazoline in tetrahydrazoline eye drops for the 12 samples (6 samples from each analyst) was 0.9% indicating a good intermediate precision of the method.

3.2.4. Selectivity (stability indicating evaluation)

Selectivity of the method was demonstrated by enhancing degradation of tetrahydrazoline under stress conditions (acid and base hydrolysis and oxidation), to show that tetrahydrazoline is separated from possible degradation products of tetrahydrozoline resulted from stress condition. Results have shown that tetrahydrozoline is stable in hydrogen peroxide solution (it gives no degradation products). Furthermore, it was found that tetrahydrozoline was stable when standard solution of it was stored at room temperature or in oven at 60 °C for one week. On the other hand, tetrahydrozoline was not stable in acidic and basic solutions; about 40% and 35% of tetrahydrozoline was degraded in hydrochloric acid solution and sodium hydroxide solution, respectively. However, no degradation products were detected.

3.2.5. Robustness

Robustness of the current method was investigated by analyzing three samples of tetrahydrozoline in eye drop formulations using the same chromatographic conditions set forth in method development but (a) using flow rate 0.8 and 1.2 mL/min instead of 1.0 mL/min; (b) detection wavelength 235 and 245 nm instead of 240 nm, and (c) volume fraction of acetonitrile is 18% and 22% instead of 20%. RSD of the percentage of tetrahydrozoline under these conditions is calculated to be less than 1% (Table 2).

After successful development and validation of this method, it was employed for analysis of tetrahydrozoline in eye drop formulations as shown in Fig. 2.

Table 1 Accuracy (% recovery) of tetrahydrozoline in eye drop formulation at three concentration levels.

Tetrahydrozoline concentration (mg/mL)	Accuracy (% recovery)				RSD (%)
	Sample 1	Sample 2	Sample 3	Mean	
0.025	100.9	101.3	100.6	100.9	0.35
0.050	101.1	101.0	100.6	100.9	0.26
0.075	99.8	100.7	101.0	100.5	0.62

Table 2 Robustness testing of the tetrahydrozoline.

Parameter	Content of tetrahydrozoline assay (%)				RSD (%)
	Sample 1	Sample 2	Sample 3	Mean	
Flow rate (mL/min)					
0.8	101.2	101.6	101.4	101.4	
1.0	100.3	100.6	100.1	100.3	0.67
1.2	99.7	100.8	99.9	100.1	
Acetonitrile (%)					
18	101.1	100.2	100.7	100.7	
20	99.8	101.1	100.9	100.6	0.70
22	99.1	100.1	99.7	99.6	
Wavelength (nm)					
235	99.8	101.1	100.8	100.6	
240	100.3	101.1	100.6	100.7	0.52
245	101.6	100.7	101.0	101.1	

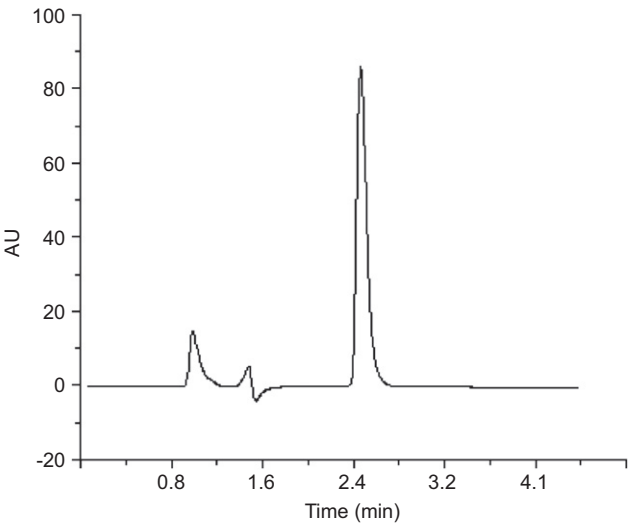

Figure 2 Chromatogram of tetrahydrozoline in eye drop formulation. Mobile phase: acetonitrile/potassium dihydrogen phosphate buffer—pH 3.0 (20:80, v/v), flow rate—1.0 mL/min, injection volume—20 µL. Column: C_8, 5 µm and 12.5 cm—length, 4.6 mm—inner diameter, UV detection: 240 nm. Peak asymmetry and theoretical plates of tetrahydrozoline peak are 0.98 and 3100, respectively.

4. Conclusion

A simple, accurate, precise, and stability-indicating HPLC method was developed and validated for the routine analysis of tetrahydrozoline in eye drop formulations. The results

of stress testing undertaken according to the International Conference on Harmonization guidelines reveal that the method is selective and stability-indicating.

Conflict of interest statement

We (the authors of this manuscript) have no financial and personal relationships with other people or organizations that could influence our work.

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